Synthesis and properties of photo-reactive antisense oligonucleotides containing 2′-O-psoralen-conjugated adenosine

Maiko Higuchi1, Asako Yamayoshi2, Akio Kobori1, Tetsuji Yamaoka3 and Akira Murakami1
1Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 602-0841, Japan, 2Institute of Materials Chemistry and Engineering, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan and 3Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

ABSTRACT

In order to selectively regulate mRNA having a point mutation, the photo-reactive antisense oligonucleotides were developed. Two types of photo-reactive oligonucleotides containing adenosine whose 2′-OH was modified with 4,5′,8-trimethylpsoralen (psoralen) were synthesized (2′-Ps-oligo). One contains psoralen via a methylene linkage (2′-Ps-met), and the other via an amidomethylene linkage (2′-Ps-amd). 2′-Ps-oligos were then subjected to the photo-cross-linking reaction. 2′-Ps-met cross-linked to the complementary RNA and scarcely did to the RNA having a single mismatch base. Contrarily, 2′-Ps-amd did not cross-link to both RNA strands. These results suggest the structure of the linkage might affect the efficiency of the photo-cross-linking.

INTRODUCTION

Through the identification of human genome sequence and the development of molecular biology, it has found that various serious diseases were caused by the consequence of a single point mutation in the coding region. For example, ras oncogene having a single point mutation in the coding region is responsible for the transformation [1,2]. The mutation affects the cellular proliferation and induces tumorigenic properties. It is desired to inhibit selectively the expression of disease-causing mutation without affecting normal genes. Traditional drugs have certain limitations for such diseases because of the lack of the sequence specificity. The antisense strategy which inhibits gene expression in a sequence specific manner might be suitable for the purpose.

We designed new photo-reactive antisense oligonucleotides to suppress the expression of mRNA having a single point mutation and 4,5′,8-trimethylpsoralen (psoralen) was adopted as the photo-reactive molecule.

In our previous study, it was found that 5′-O-psoralen-conjugated oligonucleotide (5′-Ps-oligo) [3] which was complementary to HPV18 mRNA inhibited the cellular proliferation of cervical carcinoma cells (C4II) upon UV/irradiation [4]. However, there is some possibility that the

Fig. 1. Structures of 2′-Ps-oligos (2′-Ps-met and 2′-Ps-amd)

psoralen of 5′-Ps-oligo could photo-cross-link to the undesired sites of mRNA because of the flexibility of the linkage. In this study, photo-reactive oligonucleotides whose 2′-OH was modified with psoralen were designed to overcome the problem.

RESULTS AND DISCUSSION

We designed the photo-reactive antisense molecule so as to cross-link to pyrimidine base at the site of the point mutation in the target RNA, and so as not to randomly cross-link to the pyrimidine bases. The antisense molecules possess a psoralen derivative at 2′-position via either a methylene linkage (2′-Ps-met) or an amidomethylene linkage (2′-Ps-amd). The conformational calculation of the hybrid between 5′-Ps-oligo or 2′-Ps-oligo and its complementary RNA was carried out using AMBER* force field in MacroModel ver.8.0. It was demonstrated that both psoralens of 2′-Ps-met and 2′-Ps-amd intercalated only between the designated base pairs. Contrarily, the psoralen of 5′-Ps-oligo intercalated between the designated base pairs and also between the neighboring base pairs. This might cause the undesired cross-linking. The calculated distances between the double bond of the pyrimidine base 5-6 and the double bond of psoralen 3-4 were 5.7 Å in the 2′-Ps-met and 4.0 Å in the 2′-Ps-amd.
Two types of 2'-O-psoralen-conjugated adenosine phosphoramidites were synthesized. The introduction of psoralen derivatives to the 2' position was achieved in two ways. (I) 4'-Chloromethyl-4,5',8-trimethylpsoralen was selectively introduced to 2'-position of adenosine whose 2'-OH and 3'-OH were activated by sodium hydride. (II) 4'-Aminomethyl-4,5',8-trimethylpsoralen (AMT) was introduced to 2'-position of N'-benzoyl-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)adenosine adopting the carbonyldiimidazole (CDI) activation protocol (Scheme 1) [5]. The reaction of 2 with CDI in THF gave 2'-O-(imidazolyl-1-y-carbonyl)-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)adenosine 3 in nearly quantitative yield. Subsequently, 3 was treated with AMT in THF to give 4 and 4 was isolated by column chromatography (87%). These 2'-O-psoralen-conjugated adenosine derivatives (Aps) were introduced to oligonucleotide by the solid phase synthesis protocol. The sequence of the Ps-oligo was d(CCTCGACAPSACCGCAT), which was complementary to codon 12 of K-ras mRNA surrounding region.

2'-Ps-oligos were then subjected to the photo-cross-linking reaction with their target RNAs. The reactions were analyzed by RPLC and denaturing PAGE. 5'-Ps-oligo cross-linked to both its complementary RNA and RNA having a single mismatch base. It means that 5'-Ps-oligo could cross-link to the undesired site of RNA. On the other hand, 2'-Ps-met cross-linked to its complementary RNA upon UVA-irradiated, but scarcely cross-linked to the RNA having a single mismatch base. These results suggest that 2'-Ps-met could selectively cross-link to the site of a single base mutation of RNA.

CONCLUSION

We reported the synthesis of photo-reactive antisense oligonucleotides containing 2'-O-psoralen-conjugated adenosine. Photo-cross-linking reaction studies indicated 2'-Ps-met has higher selectivity than 5'-Ps-oligo, and 2'-Ps-met becomes a powerful antisense molecule to inhibit the expression of mRNA.

REFERENCES